(Abroad・Domestic) Official trip report form (Student) 2014/12/03 (Year/Month/Day)

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| Name | Jeewan THAPA |
| Laboratory | Division of Bioresources, Research Center for Zoonosis Control |
| Year (Grade) | D2 |
| Destination | NEPAL (Kathmandu, Chitwan National Park and Pokhara) |
| Period of trip | November 04 to November 30, 2014 |
| Purpose of trip | For doing field research work for the study "Molecular epidemiology of tuberculosis (TB) in human-livestock-wildlife interface in Nepal, a one-health model from Nepal and Japan" from the support of grant in aid for graduate students. |

Summary of activities (about 800 words, provide photos, tables and figures that clearly show the activities during the period)

The main objective of the research was to collect samples from extrapulmonary (EPTB) lesions of human and suspected tuberculosis (TB) samples from livestock and wildlife to understand the molecular epidemiology of tuberculosis in human--livestock-wildlife interface. Thus, following activities were conducted to conduct grant in aid field research activities:

**1. Meeting with collaborators**

Since, sample collection is the collaborative work with the partners in Nepal; we conducted series of meeting with corresponding partners for sample collection. I joined the meeting of Prof Suzuki and Nakajima sensei with Mr Bhagwan Maharjan and Dr Bhabana Shrestha at Nepal Antituberculosis Association- German Nepal TB project (NATA-GENETUP). We are collecting EPTB samples from NATA-GENETUP and also culturing TB suspected samples from animals there. I also joined senseis during the meeting with Ms Sarita Jnawali from National Trust Nature Conservation (NTNC)-Central Zoo about future collaborative work and sample collection of wild animals. Also, I joined senseis in the meeting with Dr Basu Dev Pandey of Department of health services. Additionally, I met and discussed with officials from NTNC, Department of National Park and wildlife Conservation, Chitwan National Park, Department of livestock services and Vulture restaurants at Kaski and Chitwan about my research and sincerely requested them to help me in my future work.


Fig1. Meeting with TB collaborators


Fig 2. Meeting at Central Zoo

**2. Collection of EPTB samples from human**

To understand the zoonotic tuberculosis in Nepal, We have been targeting EPTB samples in humans. Since, NATA-GENETUP is one of the only two WHO reference laboratories in Nepal, so, we are collecting samples from there. Epidemiological information such as age, sex, location of patients and body parts of sample is recorded. DNA is collected from the obtained culture samples. During this study period, epidemiological information of 247 suspected EPTB samples were collected. Out of 247 cultured samples that were submitted from September 2013 to September 2014, 27 samples were culture positive for TB. Out of 27 culture positive samples, I extracted DNA from 14 samples and sent to Hokkaido University Japan whereas 7 DNA samples were examined before and 6 samples were not available for DNA extraction.

Fig 3. Collecting epidemiological information of EPTB isolates.

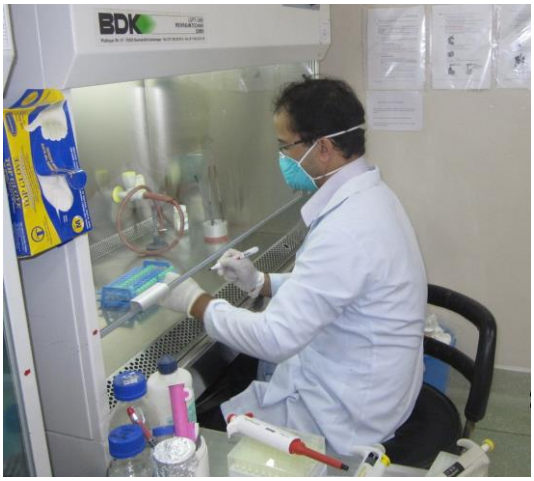


Fig 4. DNA extraction from EPTB isolates.

It was noticed that around 22 EPTB samples were negative at culture but had positive direct DNA detection genetic tests like Gene expert (16 samples) and Hains test (6 samples). Also, 16 samples from total of 247 EPTB samples were only microscopic (smear test) positive with culture negative and no genetic analysis information. This information suggests that these samples might have very less bacteria load or dead bacteria because of

treatment.

**Table 1. Description of 14 samples brought on Nov 2014**

| SN | Age | Sex | EPTB samples | Microscopy | Culture |
|---|---|---|---|---|---|
| 1 | 23 | F | pus | 1+ | 1+ |
| 2 | 73 | F | Pus | Neg | 1+ |
| 3 | 30 | M | Pus | 5AFB | 1+ |
| 4 | 33 | M | Pus | 1+ | 1+ |
| 5 | 23 | F | Pus | Neg | 6 col |
| 6 | 11 | F | Pus | 1+ | 1+ |
| 7 | 68 | F | Pus | Neg | 2 col |
| 8 | 27 | M | pus | 2AFB | 3 col |
| 9 | 19 | M | pus | 2+ | 3col |
| 10 | 45 | F | Pus | Neg | 1+ |
| 11 | 22 | F | Pus | Neg | 2col |
| 12 | NA | NA | pus | NA | NA |
| 13 | NA | NA | pus | NA | NA |
| 14 | NA | NA | pus | NA | NA |

**3. Tuberculosis surveillance in livestock**

During this time, our target was to perform tuberculin test in the cows that are placed at vulture restaurants at Kaski and Chitwan National Park. Many of these cows are old and thin and they are fed to vultures (big carnivore bird under protection) after they die. We wanted to know the situation of tuberculosis in those cows as we thought that those old and thin cows collected from various parts of the region could be ideal for tuberculosis testing.

Tuberclin test was performed at 10 cows and oxen at Kaski and 16 cows at Chitwan. On the first day the cervical fold of cow was measured by Vernier callipeer and tuberculin antigen was injected. Then, after 72 hours, the injection site was observed and re-measured. In this study, all the cows were negative for tuberculosis. Additionally, three cows carcass that were available, were also examined for tuberculosis lesions, no any TB lesions were observed. Thus, TB sample was not collected from livestock. Despite this fact, the staff of center informed that he had seen TB like lesion (need to be confirmed?) few time in past. So, I requested him to collect samples on my behalf and sent to NATA-GENETUP for culture. Based on this communication, I still think we might have chance of find TB samples from livestock. Thus, I think, this work needs to be properly followed and monitored in near future

for potentially sample collection.


Fig 5. Tuberculin testing of cows at vulture restaurant.


Fig 6. Scenario of feeding dead cow after de-skinning to vultures. PM of this cow was done to see TB lesions.

**4. Sample collection from wild animals of Central Zoo**

A sample was collected from a Black Buck that died with suspected tuberculosis lesion from Central Zoo. I transported this sample to NATA-GENETUP and cultured for TB. Beside this, I also culture a sample from a crane although it did not have any TB lesions. In our previous study, we have isolated *Mycobacterium orygis* from other two animals namely blue bull and spotted deer. We would further like to continue samples to understand tuberculosis transmission dynamics and molecular epidemiology of isolates.

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| Approval of supervisor | Institution・Official title・Name :<br>Hokkaido University<br>Research Center for Zoonosis Control<br>Professor<br>Yasuhiko SUZUKI 印 |

※1 Send the electronic file to the Leading School section, International Affairs Office, also submit the original print out with seal of supervisor to the Leading School section, International Affairs Office.

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